

# Variable Mate-Guarding Time and Sperm Allocation by Male Snow Crabs (*Chionoecetes opilio*) in Response to Sexual Competition, and their Impact on the Mating Success of Females

AMÉLIE RONDEAU<sup>1,\*</sup> AND BERNARD SAINTE-MARIE<sup>2,†</sup>

<sup>1</sup>*Institut des sciences de la mer de Rimouski (ISMER), Université du Québec à Rimouski, 310 allée des Ursulines, Rimouski, Québec G5L 3A1, Canada; and* <sup>2</sup>*Division des invertébrés et de la biologie expérimentale, Institut Maurice-Lamontagne, Pêches et Océans Canada, 850 route de la Mer, C.P. 1000, Mont-Joli, Québec G5H 3Z4, Canada*

**Abstract.** Two laboratory experiments investigated mate guarding and sperm allocation patterns of adult males with virgin females of the snow crab, *Chionoecetes opilio*, in relation to sex ratio. Although females outnumbered males in treatments, operational sex ratios were male-biased because females mature asynchronously and have a limited period of sexual attractiveness after their maturity molt. Males guarded females significantly longer as the sex ratio increased: the mean time per female was 2.9 d in a 2♂:20♀ treatment compared to 5.6 d in a 6♂:20♀ treatment. Female injury and mortality scaled positively to sex ratio. Males that guarded for the greatest number of days were significantly larger, and at experiment's end had significantly smaller vasa deferentia, suggesting greater sperm expense, than males that guarded for fewer days. In both experiments, the spermathecal load (SL)—that is, the quantity of ejaculate stored in a female's spermatheca—was independent of molt date, except in the most female-biased treatment, where it was negatively related. The SL increased as the sex ratio increased, mainly because females accumulated more ejaculates. However, similarly sized males had

smaller vasa deferentia and passed smaller ejaculates, such that, at a given sex ratio, the mean SL was 55% less in one experiment than in the other. Some females extruded clutches with few or no fertilized eggs, and their median SL (3–4 mg) was one order of magnitude smaller than that of females with well-fertilized clutches (31–50 mg), indicating sperm limitation. Males economized sperm: all females irrespective of sex ratio were inseminated, but to a varying extent submaximally; each ejaculate represented less than 2.5% of male sperm reserves; and no male was fully exhausted of sperm. Sperm economy is predicted by sperm competition theory for species like snow crab in which polyandry exists, mechanisms of last-male sperm precedence are effective, and the probability that one male fertilizes a female's lifetime production of eggs is small.

## Introduction

Intrasexual competition for mates is a fundamental characteristic of sexual reproduction (Trivers, 1972). The intensity of sexual competition depends mostly on the operational sex ratio (OSR), which is the number of sexually active males relative to the number of fertilizable females at a given site and time (Emlen and Oring, 1977). In many animal species, females care for progeny and are only briefly and infrequently receptive, giving them a much smaller potential rate of reproduction than males. This causes the OSR to be skewed toward males, a tendency that may be exacerbated if females become receptive asynchronously. As a result, sexual competition is often more intense among males than among females; to enhance their repro-

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\* Present address: Division des poissons marins, Centre des pêches du Golfe, Pêches et Océans Canada, C.P. 5030 Moncton, Nouveau Brunswick E1C 9B6, Canada.

† To whom correspondence should be addressed. E-mail: Sainte-MarieB@dfo-mpo.gc.ca

*Abbreviations:* CW, carapace width; GT, guarding time per female; ESR, effective sex ratio; OSR, operational sex ratio; SL, spermathecal load; VDW, vasa deferentia weight.

ductive success, males may express flexible behaviors, including mate guarding and judicious allocation of sperm (Trivers, 1972; Ridley, 1983; Clutton-Brock and Parker, 1992).

Precopulatory mate guarding is taxonomically widespread, albeit particularly common in the Crustacea, and it may serve to monopolize a female until she is fertilizable (Parker, 1974; Ridley, 1983). Postcopulatory mate guarding occurs mostly in species with direct sperm transfer, and it may help to ensure paternity for the guarding male by preventing rival males from inseminating the female until she has fertilized her eggs or is no longer receptive (Parker, 1970; Smith, 1984). In crustacean species in which female molting and mating are intimately linked, postcopulatory mate guarding may also shield the postmolt female (and the male's reproductive investment) from predators until her shell has hardened enough to offer protection (Hartnoll, 1969; Wilber, 1989; Jivoff, 1997a). Males may vary their mate-guarding pattern in relation to competition and maximize the number of eggs gained during a breeding season by balancing the time spent guarding mates against the time spent searching for new mates (Parker, 1974; Christy, 1987). Theory predicts (Grafen and Ridley, 1983; Yamamura and Jormalainen, 1996) and observations typically confirm (see Jormalainen, 1998) that males respond to increasing sex ratio by guarding females longer.

Judicious sperm-allocation patterns have evolved in part because sperm, spermatophores, and seminal fluid can be in limited supply due to low rates or high costs of production (Dewsbury, 1982; Pitnick and Markow, 1994). Further, males may enhance their reproductive success if they adjust sperm expenditure to the perceived risk of sperm competition, which may vary as a function of sex ratio, potential for polyandry, or female mating history (Parker *et al.*, 1997). Males typically increase sperm expenditure in the presence of larger females and scale the amount of sperm allocated to females positively to the sex ratio and the risk of sperm competition (Gage, 1991; Gage and Barnard, 1996; Wedell and Cook, 1999).

Changes in male competition intensity and male mating patterns may influence female mating success. As competition becomes more intense, the risk of female injury or death may increase due to male harassment and more frequent takeover attempts (*e.g.*, Rowe *et al.*, 1994; Vepsäläinen and Savolainen, 1995). Conversely, when competition is relaxed and postcopulatory guarding is curtailed, postmolt females are more exposed to predators (Wilber, 1989; Jivoff, 1997b). A severe reduction in sperm allocation may lead to sperm limitation and loss of fecundity for females (Pitnick, 1993; MacDiarmid and Butler, 1999).

Although considerable evidence of flexible patterns of sperm allocation exists for terrestrial and freshwater animals with direct sperm transfer, very little is known of this phenomenon in their marine counterparts (Wilber, 1989; Jivoff, 1997b; Sainte-Marie *et al.*, 1997; MacDiarmid and

Butler, 1999). The present study on the snow crab (*Chionoecetes opilio*; Majidae), a marine brachyuran of the northern hemisphere, documents mate guarding and sperm allocation in relation to sex ratio for adult males with virgin females. Male mating strategies predictably are quite flexible in snow crab because the intensity of sexual competition may be highly variable among years as a result of intrinsic, circa-decadal oscillations of 1–2 orders of magnitude in the abundance ratio of adult males to virgin females. Such oscillations arise from the interaction of multiyear variations in year-class strength and of sexual dimorphism in age at maturity, leading to temporally staggered recruitment patterns for adult females and males (Sainte-Marie *et al.*, 1996).

The relationships of mate-guarding time and sperm allocation to sexual competition remain undetermined for snow crab and congeners; however, other aspects of the sexual interactions of males with virgin females are very well documented in the genus *Chionoecetes*. Female snow crabs reach sexual maturity at a terminal molt, which occurs from January to April in the northwest Atlantic (see Alunno-Bruscia and Sainte-Marie, 1998). Males are attracted to pre-mature females by chemical cues (Bouchard *et al.*, 1996; Pelletier *et al.*, 1998) and then engage in courtship and precopulatory mate guarding until the female molts (Watson, 1972; Donaldson and Adams, 1989). Females usually extrude a clutch of eggs within 1–5 d of molting, whether mated or not (Paul and Adams, 1984; Sainte-Marie and Lovrich, 1994). Both fertilized and unfertilized eggs attach to the pleopods: those fertilized are incubated for up to 2 years; those not fertilized are lost within 5–6 months of attachment (Sainte-Marie, 1993; Sainte-Marie and Carrière, 1995; Moriyasu and Lanteigne, 1998). Adult males have a very high potential reproductive rate and can mate effectively with several females in rapid succession (Watson, 1972; Adams and Paul, 1983; Sainte-Marie and Lovrich, 1994). Female asynchronous molting and brief postmolt sexual attractiveness lead to male competition, and adult males that are smaller, have a softer shell, or are missing more pereopods may be displaced from females by more vigorous males (Stevens *et al.*, 1993; Sainte-Marie *et al.*, 1999). Intense male competition also favors polyandry, and female snow crabs during their first breeding period may accumulate in their spermathecae the ejaculates of up to six males (Urbani *et al.*, 1998). Multiple (different males) and repeated (same male) copulations can happen before or shortly after the first egg clutch is extruded (Sainte-Marie *et al.*, 1997, 1999). When multiple mating takes place before oviposition, last-male sperm precedence usually occurs through a combination of sperm displacement and postcopulatory mate guarding (Urbani *et al.*, 1998; Sainte-Marie *et al.*, 2000). The amount of ejaculate stored by females is independent of mate body size (Adams and Paul, 1983; Sainte-Marie and Lovrich, 1994) but is positively related to



number and duration of copulations, which are hypothesized to be influenced by competition intensity (Sainte-Marie *et al.*, 1997).

The present laboratory study of mating behavior in snow crab was guided by three hypotheses: (i) larger males guard more than smaller males, (ii) mate guarding lasts longer at higher than at lower sex ratios, and (iii) females store more sperm as the sex ratio increases. We also measured the effects that changes in male competition intensity and reproductive investment have on the mating success of females. Two mating experiments were conducted to determine if sperm allocation patterns in relation to competition were the same whether sex ratio was manipulated by varying the density of females or of males. Experiments used female-biased treatments to explore the potential for sperm limitation, a major concern where snow crab fisheries remove only large adult males (Kruse, 1993; Elnor and Beninger, 1995).

## Materials and Methods

### Collection of crabs

Crabs were collected in October of 1996 and 1997 in the Saint Lawrence Estuary (48°33'N, 68°23'W), eastern Canada. The carapace width (CW) of all crabs and the right chela height of males were measured to the nearest 0.1 mm, using a vernier caliper as described in Sainte-Marie and Hazel (1992). Only immature females larger than 40 mm CW were kept, because they are more likely to reach maturity at their next molt. Males retained were adults of 80 to 115 mm CW, at the mid-range of the 40–155 mm CW distribution for adult males, with shells of intermediate condition (*i.e.*, hard exoskeleton with light epibiont fouling). This shell condition prevails from about 8 months to 3 years after the male's terminal molt to adulthood (Sainte-Marie *et al.*, 1995) and coincides with peak sexual competitiveness (Sainte-Marie *et al.*, 1999).

Selected females and males were brought to the Maurice Lamontagne Institute and placed in separate tanks supplied with fresh running seawater. Photoperiod was controlled to reflect the natural light cycle. Crabs were fed excess thawed shrimp (*Pandalus borealis*) and Atlantic herring (*Clupea harengus*) on a semi-weekly basis from the time of capture to the end of the experiments.

### Mating experiments

Mating experiments were conducted in 1997 and in 1998. Crabs were used only in the winter after collection. Thus the time elapsed between collection and use in experiments was similar in both years.

The 1997 experiment ran from 31 January to 4 April (64 d) in nine tanks, each with a bottom surface area of 1.14 m<sup>2</sup>

(390 l). Tanks received fresh running seawater with ranges of temperature (−0.5 to 1.5 °C) and salinity (24.6‰ to 30.2‰) over the duration of the experiment that represented natural conditions for snow crab. Sex ratio was controlled by varying the number of females for a constant number of males; treatments had male-to-female ratios of 2:10 ( $n = 3$  replicates), 2:20 ( $n = 3$ ), and 2:30 ( $n = 3$ ). Female and male crabs were allotted to tanks so that their respective size distributions were as similar as possible among all replicates. Immature females were missing no pereopods at the time they were placed in tanks. Some males were missing 1–2 walking legs, but all had two chelae. Excess crabs were held in reserve tanks. Tanks were checked twice daily for the presence of molting females, and any exuvium was removed and measured to determine premolt CW. Several days after a female had molted to maturity and mating behavior had ceased, the female was identified with a numbered plastic tag tied around the coxopodite of a pereopod. During the experiment, dead females were replaced by a female of similar CW and same reproductive stage taken from the reserves. Pre-mature females are called "pubescent," females that have molted to maturity but not yet extruded eggs are called "nulliparous," and females that have laid their first clutch of eggs are called "primiparous." Substitute females were also tagged. At the experiment's end, the CW of primiparous females was measured.

The 1998 experiment ran from 25 January to 30 March (65 d) in 12 tanks, each with a bottom surface area of 2.23 m<sup>2</sup> (740 l). Tanks were supplied with fresh running seawater ranging from −0.3 to 1.8 °C and from 24.4‰ to 31.3‰ salinity over the duration of the experiment. Sex ratio was controlled by varying the number of males for a constant number of females; male-to-female ratios in treatments were 2:20 ( $n = 4$  replicates), 4:20 ( $n = 3$ ), 6:20 ( $n = 4$ ), and 10:20 ( $n = 1$ ). The 2♂:20♀ treatment was common to the 1997 and 1998 experiments. Methods were identical to 1997, except for the following. All males were intact and each was identified with a water-resistant, numbered label fixed to the dorsum. All females in one replicate each of the 2♂:20♀ and 6♂:20♀ treatments were similarly identified with a label that bore a letter. Labels were large enough to be read from a distance but did not impede molting or mating. Each day we determined the number of males that were guarding females and recorded specific mating associations in the two tanks where all crabs were labeled. Guarding males were those grasping a female or copulating.

The high densities of crabs in our treatments, reaching up to 28 crabs m<sup>-2</sup>, are not unrealistic. Majid crabs are notorious for their gregarious behavior during the mating season (*e.g.*, DeGoursey and Auster, 1992), and densities of 100 crabs m<sup>-2</sup> have been documented for *Chionoecetes bairdi* (Stevens *et al.*, 1994).

### Male reproductive effort

The weight of vasa deferentia (VDW), which include the storage areas for spermatophores and seminal fluid, was determined at the end of experiments to evaluate sperm depletion as a potential indicator of male reproductive effort. Males in the 1997 and 1998 experiments and 16 reserve males (unmated = controls) of 1998 were killed and were injected with, and immersed in, 4% seawater-diluted formalin. Males were subsequently dissected and their vasa deferentia were removed, blotted, and weighed to the nearest milligram.

In 1998 we estimated guarding time for individual females (GT, in days) in each replicate as

$$GT = \sum n\delta_i / N\varphi_m$$

where  $n\delta_i$  is the number of males that were guarding a female on the  $i_{th}$  day of the experiment and  $N\varphi_m$  is the number of females having reached maturity at experiment's end. GT includes both precopulatory and postcopulatory mate guarding, which could not be dissociated under the present experimental conditions. In the two replicates where all crabs were labeled, we determined for each female the time elapsed between the occurrence of first grasping and the maturity molt, and the total number of days she was guarded.

### Female mating success

Female mating success was assessed for primiparous females (excluding primiparous substitutes) at the end of experiments by measuring percent fertilized eggs per clutch, clutch weight, and spermathecal load (SL). Injury and death are also components of female mating success, so the number of missing pereopods and the percent mortality of nulliparous and primiparous females were compiled for each replicate.

The percentage of fertilized eggs in a clutch was estimated from a sample of eggs taken from random locations throughout the clutch while the female was alive. Following Carrière (1995), eggs were processed to highlight nuclei for determination of the proportion of divided (= fertilized) eggs per sample. Briefly, eggs were fixed for 1 h in a solution of 97% glucamine-acetate (GA) buffer, 2% formalin, and 1% Triton, and then rinsed in GA buffer. Eggs were then stained for 1 h in a solution of 0.5  $\mu$ g Hoechst dye per ml of GA buffer, and were rinsed twice and preserved in GA buffer at 4 °C. A sample typically contained 200–2400 eggs, and divided and undivided eggs were counted under epifluorescent microscopy. Because eggs develop slowly at cold temperatures, their fertilization status cannot be accurately determined before they are 20 d old (Rondeau, 2000), so we sampled only primiparous females older than 20 d

postmolt, reasonably assuming no delay between molt and oviposition.

The female was killed after her eggs were sampled. The remaining clutch was removed, by severing the base of the inner ramus of each pleopod, and preserved in 99% ethanol. The weight of the blotted clutch was measured to the nearest milligram and then was adjusted using correction factors in Rondeau (2000) to account for enclosed pleopod rami and for removed eggs. The right spermatheca was extracted from the female and preserved in 4% seawater-diluted formalin. Subsequently, SL was determined by peeling away the wall of the spermatheca and weighing its blotted content to the nearest 0.1 mg. Total ejaculate stored by a female can be estimated by doubling the SL because delivery of sperm is usually balanced between the two spermathecae (Sainte-Marie and Lovrich, 1994). Females were considered for analyses of SL only if they survived for 3 days after their maturity molt, to ensure they had the opportunity to fully realize their mating potential. After determination of SL, we estimated the number of sperm stored in some spermathecae from the 1998 experiment. To provide an even distribution over the range of SLs for each treatment, 10 spermathecae from each of the 2 $\delta$ :20 $\varphi$  and 10 $\delta$ :20 $\varphi$  treatments were selected before sperm were counted. Our method for counting sperm was to homogenize the spermathecal contents, dilute the homogenate in seawater to a known volume, enumerate the sperm in replicate hemacytometers, and then extrapolate the average sperm count to the total volume (Adams and Paul, 1983; Sainte-Marie and Lovrich, 1994).

### Data analysis

For each replicate, the effective sex ratio (ESR) was calculated as

$$ESR = N\delta / (N\varphi_m + N\delta)$$

where  $N\delta$  is the number of males and  $N\varphi_m$  is the number of mature females available to males during the experiment. Following Emlen and Oring (1977), the operational sex ratio (OSR) was calculated as

$$OSR = \sum [N\delta / (n_i\varphi_j + N\delta)] / D$$

where  $N\delta$  is the number of males,  $n_i\varphi_j$  is the number of fertilizable females on the  $i_{th}$  day, and  $D$  is the duration of the experiment in days. To calculate OSR, we somewhat arbitrarily used a fertilizable period of 3 d starting at the maturity molt. Our choice is justified by the fact that when males are present, female snow crabs usually are inseminated and extrude eggs within 6–24 h of molting (Watson, 1972; Sainte-Marie and Lovrich, 1994) but may continue to mate for about 2–3 d after oviposition. However, although males may compete intensively for nulliparous (pre-oviposition) females, there is little or no male competition for females after oviposition (Sainte-Marie *et al.*, 1997, 1999).



Table 1

Mean  $\pm$  standard deviation of effective sex ratio (ESR) and of operational sex ratio (OSR, calculated assuming a 3-d fertilizable period for females) for treatments in the 1997 and 1998 mating experiments with snow crab

1997			1998		
Treatment	ESR	OSR	Treatment	ESR	OSR
2♂:30♀	0.06 $\pm$ 0.00	0.69 $\pm$ 0.03	2♂:20♀	0.11 $\pm$ 0.03	0.81 $\pm$ 0.04
2♂:20♀	0.09 $\pm$ 0.01	0.78 $\pm$ 0.03	4♂:20♀	0.18 $\pm$ 0.01	0.89 $\pm$ 0.00
2♂:10♀	0.18 $\pm$ 0.03	0.87 $\pm$ 0.00	6♂:20♀	0.29 $\pm$ 0.06	0.93 $\pm$ 0.02
			10♂:20♀	0.39	0.94

and thus some uncertainty exists as to how the fertilizable period should be defined for calculation of OSR. Given this uncertainty, we preferred to use ESR as a basis for comparison of male reproductive effort and female mating success across treatments. Both ESR and OSR can take on values ranging from 0 (no male) to 1 (no female), with 0.5 representing a balanced sex ratio.

For univariate analyses, we used the mean, standard deviation, and *t* test or analysis of variance (ANOVA) for description and sample comparisons of normally distributed and homoscedastic data (raw or transformed). Otherwise, the median and Mann-Whitney test were used. One-tailed tests were performed when the mean or median was expected *a priori* to be greater in one sample than in the other. Correlation analysis examined trends between pairs of variables such as female size, molt date, guarding time, and SL. Functional relationships between two variables were investigated by regression analysis. When relating VDW or SL to ESR, we used the replicate's mean or median rather than individual values of the dependent variable so that each replicate weighted the regression equally. Analysis of covariance (ANCOVA) was used to compare clutch weight among treatments with CW as the covariate, and VDW and SL between experiments with ESR as the covariate. The assumption of homogeneity of slopes was met if there was no significant interaction between factor and covariate (Sokal and Rohlf, 1995).

In primiparous female snow crabs, SL measured shortly after the mating period is the sum of individual ejaculates received from single or multiple mates, less the amount of ejaculate expended at fertilization (Sainte-Marie *et al.*, 1997). Frequency distributions of  $\log_{10}$  SL for replicated treatments were graphed and analyzed using the mixture distribution method of MacDonald and Pitcher (1979) in an attempt to resolve modes representing one or more accumulated ejaculates.

## Results

### Size of crabs, molting schedule, and operational sex ratio

Immature females ranged from 45.5 to 66.2 mm CW in the 1997 experiment and from 44.2 to 60.5 mm CW in the

1998 experiment; males were 80.4–111.7 mm CW in 1997 and 81.0–113.0 mm CW in 1998. The mean CW of immature females was homogenous among replicates and treatments in each experiment (two-way ANOVA,  $F_{8,163} = 0.18$ ,  $P = 0.993$  in 1997 and  $F_{11,156} = 1.06$ ,  $P = 0.400$  in 1998), but differed (ANOVA,  $F_{1,338} = 20.69$ ,  $P < 0.001$ ) between 1997 (53.8 mm) and 1998 (51.7 mm). The mean CW of males was homogenous among replicates and treatments in 1997 ( $F_{6,9} = 0.68$ ,  $P = 0.671$ ) and 1998 ( $F_{8,41} = 0.32$ ,  $P = 0.953$ ), and was similar ( $F_{1,69} = 2.74$ ,  $P = 0.103$ ) between 1997 (94.4 mm CW) and 1998 (98.4 mm CW).

The proportion of immature females that molted was 95.6% in the 1997 experiment and 70.0% in the 1998 experiment, and all but two moltees achieved maturity. A negative correlation between premolt CW and molt date in 1997 ( $r = -0.15$ ,  $n = 186$ ,  $P = 0.037$ ) and 1998 ( $r = -0.26$ ,  $n = 168$ ,  $P < 0.001$ ) indicated that larger females tended to molt earlier. The first female molt occurred on day 2 and day 5 of the 1997 and 1998 experiments, respectively, and molting continued until the end of each experiment. The cumulative number of molts followed a logistic pattern over time, and about 75% of molts in each treatment occurred over a period of about 25 d. After fitting and comparing logistic regressions, Rondeau (2000) found that 50% of total molts in 1997 occurred on day  $20.8 \pm 1.0$  d in the least female-biased treatment (2♂:10♀), significantly sooner than in treatments with 2♂:20♀ (day  $26.2 \pm 0.7$ ) and 2♂:30♀ (day  $26.8 \pm 0.6$ ). In 1998, females also molted sooner in the least female-biased treatment (10♂:20♀, day  $33.9 \pm 1.9$ ) than in the 6♂:20♀, 4♂:20♀, and 2♂:20♀ treatments (day  $43.7 \pm 0.7$  to  $44.3 \pm 0.8$ ). ESR and OSR values paralleled male-to-female treatment ratios, but OSR was always biased toward males (Table 1). If the fertilizable period is taken to be 1 d instead of 3 d—to reflect only the usual time between female molt and oviposition when males compete to inseminate a female—then depending on treatment, OSR ranged from 0.87 to 0.96 in 1997 and from 0.93 to 0.98 in 1998.

### Female pereopod loss and mortality

Most injury or death of females occurred while they were in the soft postmolt condition. In both experiments, the proportion of primiparous females that was missing 0, 1–2,

Table 2

Percentage of primiparous female snow crabs missing 0, 1–2, or 3 or more pereopods by replicated sex-ratio treatment in the 1997 and 1998 mating experiments ( $n$  = number of primiparous females)

Missing pereopods	Treatments					
	1997			1998		
	2♂:30♀	2♂:20♀	2♂:10♀	2♂:20♀	4♂:20♀	6♂:20♀
0	56.8	33.3	53.6	50.8	38.0	9.6
1–2	40.9	41.0	39.3	36.9	38.0	46.2
≥3	2.3	25.7	7.1	12.3	24.0	44.2
$n$	88	39	28	65	50	52

Females were intact at start of experiment. The number of females in each class of missing pereopods was not independent of treatment ( $G$ -test of independence:  $P < 0.05$  for both years). Females from one 2♂:20♀ replicate of 1997 were excluded because of incomplete information on number of missing pereopods.

or ≥3 pereopods differed significantly among treatments (Table 2). In 1998, the number of missing pereopods increased relative to the number of males and the sex ratio. In 1997, however, the number of males was held constant and there was no clear pattern between the number of missing pereopods and the sex ratio or female density. Mean mortality of combined nulliparous and primiparous females increased with increasing sex ratio. In the 1997 experiment, mortality reached 6%, 18%, and 20% in the 2♂:30♀, 2♂:20♀, and 2♂:10♀ treatments, respectively. One replicate in the 2♂:20♀ treatment was excluded from computation of mortality because combined nulliparous and primiparous mortality was high (50%). Dead females in this peculiar replicate were shredded, suggesting that one or both males were particularly aggressive. Mortality in the 1998 experiment was 15%, 20%, and 35% in the 2♂:20♀, 4♂:20♀, and 6♂:20♀ treatments, respectively.

#### Male dominance and mate-guarding patterns

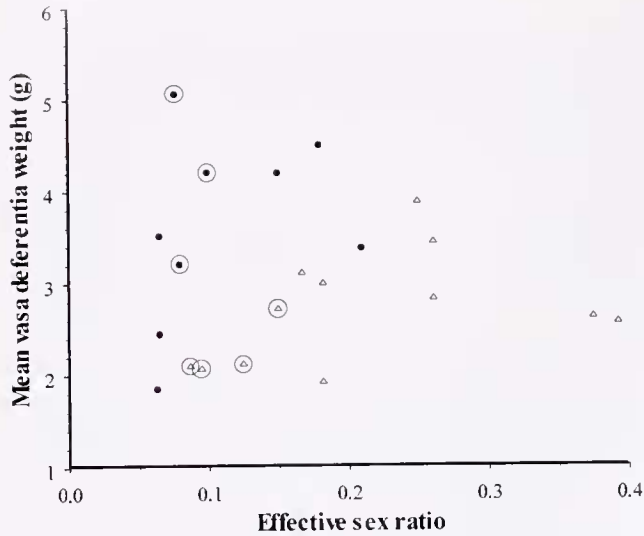
For the 1998 experiment, males were separated *a posteriori* into two groups based on the total number of guarding days accumulated by each male over the course of the experiment. In each replicate, the male that guarded for the greatest number of days was categorized as "dominant" and the other male (or males) was considered to be "subordinate." Dominant males (mean CW = 102.4 mm,  $n = 12$ ) were as expected larger (one-tailed  $t$  test,  $t = 1.74$ ,  $P = 0.049$ ) than subordinate males (mean CW = 97.3 mm,  $n = 42$ ). The average number of days that dominant males guarded in the 1998 experiment did not decline with increasing number of males per treatment (Table 3). Moreover, the decrease in the contribution of dominant males to total number of guarding days was not proportional to the increase in number of males per treatment (Table 3).

Table 3

Cumulative number of guarding days by males, and mean  $\pm$  standard deviation of guarding time for individual females (GT), in relation to sex ratio in the 1998 mating experiment with snow crab

Treatment	$n$	Number of guarding days by male type			Contribution of dominant male to sum of guarding days by all males			Mean GT
		(a) Dominant sum	Dominant mean	(b) All males sum	Observed	Expected	$G_{adj}$ , $P$	
2♂:20♀	4	88	22.0 $\pm$ 6.1	166	0.53	0.50	0.60, >0.05	2.9 $\pm$ 1.0
4♂:20♀	3	52	17.3 $\pm$ 5.9	124	0.42	0.25	16.87, <0.001	3.1 $\pm$ 0.6
6♂:20♀	4	93	23.2 $\pm$ 8.0	251	0.37	0.17	59.83, <0.001	5.6 $\pm$ 1.9
10♂:20♀	1	18	18	86	0.21	0.10	8.93, <0.01	6.5

The sum of guarding days for the dominant male (a) and for all males (b) in all replicates ( $n$ ) of each treatment are shown. The dominant male, for which mean  $\pm$  standard deviation of guarding days by treatment are given, represents the male that guarded for the greatest number of days in each replicate. Considering only replicated treatments, there was no effect of sex ratio on mean number of guarding days by the dominant male (ANOVA,  $F_{2,8} = 0.69$ ,  $P = 0.528$ ) and a significant effect of sex ratio on mean GT (ANOVA,  $F_{2,8} = 6.97$ ,  $P = 0.018$ ). Observed contribution of dominant males to sum of guarding days for all males is the quotient of (a) over (b); expected contribution of dominant males is the quotient of one over the number of males per treatment. The  $G$ -test with William's correction ( $G_{adj}$ ) verified whether observed contribution departed significantly ( $P$  = probability) from expected contribution.



**Figure 1.** Mean vasa deferentia weight of male snow crabs at end of 1997 (●) and 1998 (△) mating experiments in relation to effective sex ratio by replicate. Encircled values represent replicates of the 2♂:20♀ treatment common to both experiments. Note the outlier (effective sex ratio = 0.08, vasa deferentia weight = 5.06 g) in the 1997 experiment.

As hypothesized, GT increased as the sex ratio (and number of males) increased in the 1998 experiment (Table 3). This increase was not simply a density-dependent effect of more males haphazardly guarding any female. Indeed, based on the two replicates with all crabs labeled, females were grasped for the first time sooner (one-tailed Mann-Whitney test,  $U = 8$ ,  $P = 0.002$ ) before they molted in the 6♂:20♀ treatment (mean 9.4 d, maximum 33 d) than in the 2♂:20♀ treatment (mean 2.8 d, maximum 17 d). Also, dominant males guarded for longer continuous periods prior to the female's molt in the 6♂:20♀ treatment compared to the 2♂:20♀ treatment (one-tailed Mann-Whitney test,  $U = 3$ ,  $P = 0.007$ ), suggesting they mated fewer females as sex ratio increased. There was no correlation between the number of days a female was guarded and the date on which she molted (2♂:20♀,  $r = 0.00$ ,  $n = 18$ ,  $P = 1.000$ ; 6♂:20♀,  $r = 0.33$ ,  $n = 10$ ,  $P = 0.353$ ); therefore, females were guarded as long at the start as at the end of the experiment.

#### Sperm depletion

Sperm depletion due to mating was suggested by scatterplots showing a weak positive trend between VDW and ESR (Fig. 1), indicating that residual vasa deferentia weight tended to decline with the number of potential mating opportunities for males. ANCOVA on  $\log_{10}$ -transformed data showed that the effect of ESR on VDW was significant ( $F_{1,17} = 5.33$ ,  $P = 0.034$ ) and that year and ESR did not interact ( $F_{1,17} = 0.12$ ,  $P = 0.738$ ). The mean VDW adjusted to the overall mean ESR differed between years ( $F_{1,18} = 5.49$ ,  $P = 0.031$ ) and was 35.5% less in 1998 males

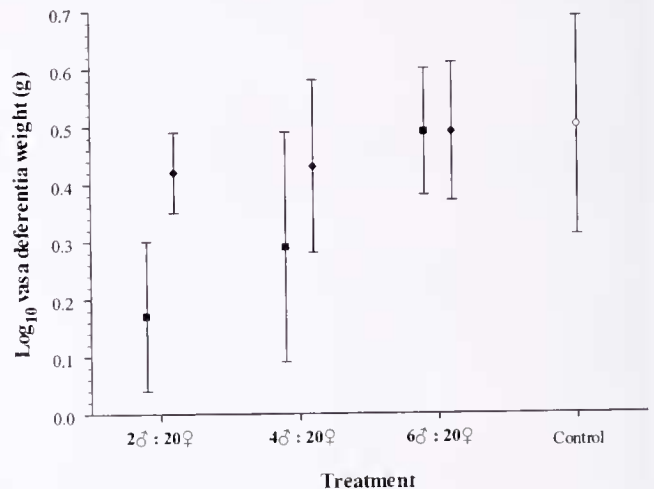
(2.45 g) than in 1997 males (3.80 g). Note the outlier in the 1997 experiment (Fig. 1), corresponding to the 2♂:20♀ replicate with unusually high female mortality.

Compelling evidence of sperm depletion was seen in the contrasting patterns of VDW for dominant and subordinate males in 1998 (Fig. 2). Two-way ANOVA excluding the sole 10♂:20♀ replicate indicated that the mean  $\log_{10}$  VDW varied with the  $\log_{10}$  ESR ( $F_{2,37} = 5.78$ ,  $P = 0.007$ ) and male hierarchy ( $F_{1,37} = 7.00$ ,  $P = 0.012$ ), but there was no interaction between the two factors ( $F_{2,37} = 2.17$ ,  $P = 0.129$ ). The mean VDW was progressively smaller as sex ratio decreased and was less in dominant than in subordinate and control males (Fig. 2), the difference between control and dominant males increasing from 2% to 66% as the sex ratio declined.

#### Spermathecal load

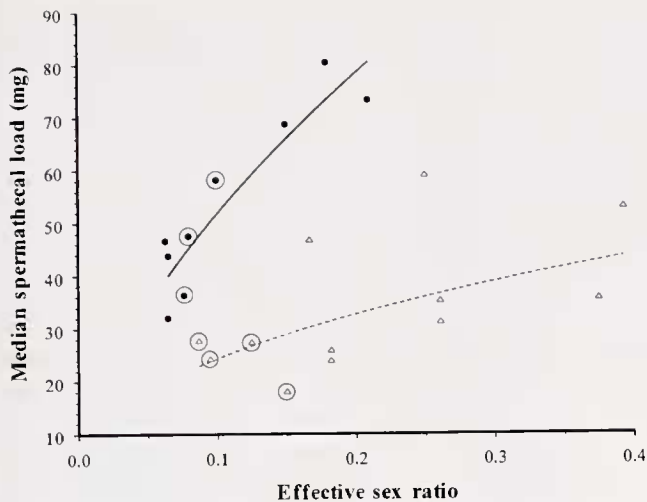
In every treatment, correlation coefficients were positive between SL and CW and negative between SL and molt date. However, the only significant coefficient was between SL and molt date ( $r = -0.31$ ,  $n = 83$ ,  $P = 0.005$ ) in the most female-biased treatment (2♂:30♀).

As hypothesized, there was a significant positive relationship between SL and ESR in both years (Fig. 3), indicating that females acquired more ejaculate as the sex ratio increased. ANCOVA on  $\log_{10}$ -transformed data showed a highly significant effect of ESR on SL ( $F_{1,17} = 17.80$ ,  $P = 0.001$ ) but no interaction between year and ESR ( $F_{1,17} = 0.50$ ,  $P = 0.488$ ). The mean SL adjusted to the overall mean ESR differed between years ( $F_{1,18} = 36.41$ ,  $P < 0.001$ ) and was 55.1% less in 1998 (27.8 mg) than in 1997 (61.9 mg).



**Figure 2.** Mean  $\pm$  standard deviation of log-transformed vasa deferentia weight of male snow crabs in relation to male mating status (dominant = ■, subordinate = ◆) in the 2♂:20♀, 4♂:20♀ and 6♂:20♀ treatments of the 1998 mating experiment and in comparison to unmated control males.





**Figure 3.** Median of right spermathecal load (SL) of primiparous female snow crabs in relation to effective sex ratio (ESR) per replicate in 1997 and 1998 mating experiments. Regressions fitted to log-transformed data are significant ( $\bullet$  1997:  $\log_{10}SL = 0.597 \cdot \log_{10}ESR + 2.310$ ,  $r^2 = 0.80$ ,  $F_{1,8} = 28.25$ ,  $P = 0.001$ ;  $\triangle$  1998:  $\log_{10}SL = 0.425 \cdot \log_{10}ESR + 1.810$ ,  $F_{1,11} = 5.18$ ,  $r^2 = 0.58$ ,  $P = 0.046$ ). Encircled values represent the 2♂:20♀ treatment common to both experiments.

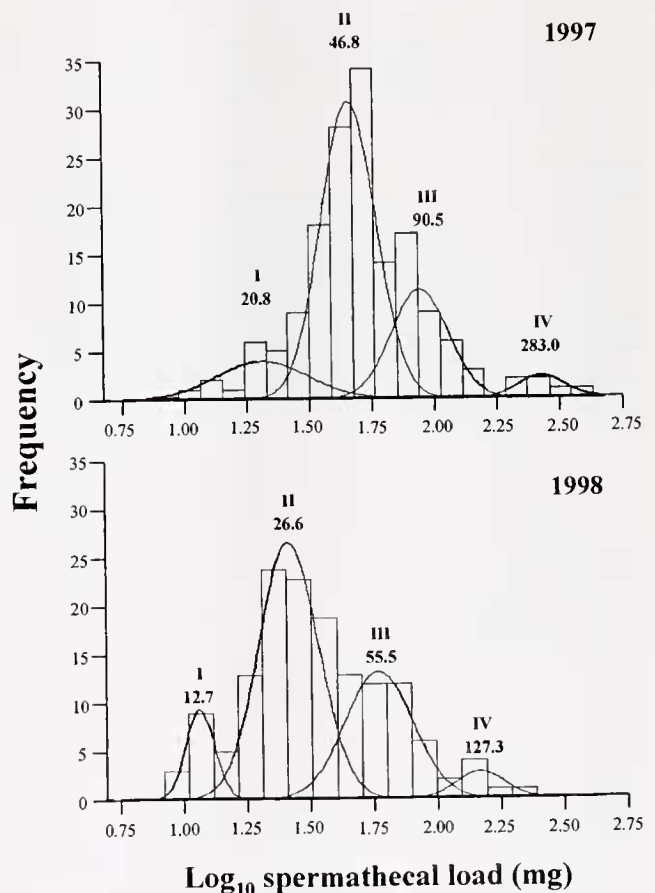
Frequency distributions of  $\log_{10}SL$  of females pooled by experiment produced a similar pattern in 1997 and 1998, consisting of four modes, of which the second was most prominent (Fig. 4). The modes are interpreted as representing spermathecal loads comprising 1, 2, 3, and 4 or more ejaculates. By differencing mean SL for two sequential modes to determine the mean size of successive ejaculates, it was apparent that ejaculates were much smaller in 1998 than in 1997, and that in any given year the size of ejaculates tended to increase with rank of introduction into the spermatheca. Mixture analysis was applied to  $\log_{10}SL$  for females pooled by treatment to determine the proportions of females receiving different numbers of ejaculates (Table 4). Two striking features emerged: in the treatments with the highest sex ratio of each experiment (2♂:10♀ in 1997 and 6♂:20♀ in 1998) no female received only one ejaculate; in contrast, in the intermediate and lower sex ratio treatments of each experiment, the proportion of females with four or more ejaculates was null in 1997 or very small in 1998. Some trends between ejaculate size and sex ratio may be biologically meaningful (e.g., the inverse relationship between size of third and fourth ejaculates and sex ratio in 1998) but must be regarded with circumspection given the small sample sizes.

Regression of sperm counts on SL with intercept forced to 0 was significant for the 2♂:20♀ and 10♂:20♀ treatments ( $r^2 \geq 0.89$ ,  $n = 10$ , and  $P < 0.001$  for each regression). Slopes did not differ significantly between the two treatments ( $F_{1,17} = 3.12$ ,  $P = 0.095$ ), so we pooled the data and produced a common regression (Fig. 5).

#### Clutch weight and percent fertilized eggs

Regression using  $\log_{10}$ -transformed data determined a positive relationship between clutch weight and female postmolt CW for each treatment of both experiments ( $r^2 \geq 0.59$  and  $P < 0.05$  for any given regression). In the 1997 experiment, the slopes of size-fecundity relationships were identical ( $F_{2,167} = 0.00$ ,  $P = 0.999$ ), but the elevations differed ( $F_{2,169} = 3.14$ ,  $P = 0.046$ ) among treatments. Mean clutch weight adjusted to overall mean CW decreased with increasing sex ratio, from 6.77 g in the 2♂:30♀ treatment to 6.47 g in the 2♂:10♀ treatment. In the 1998 experiment, size-fecundity relationships had similar slopes ( $F_{3,139} = 1.01$ ,  $P = 0.392$ ) and elevations ( $F_{3,142} = 1.59$ ,  $P = 0.194$ ) among treatments.

Percent fertilized eggs per clutch followed a dichotomous pattern: either at least 95% of the eggs were fertilized (=



**Figure 4.** Frequency distribution of right spermathecal load for primiparous female snow crabs in the 1997 and 1998 mating experiments. Four modes were fitted almost perfectly to the distributions in the 1997 ( $\chi^2 = 9.78$ ,  $P = 0.913$ ) and 1998 ( $\chi^2 = 2.78$ ,  $P = 0.999$ ) experiments using the mixture analysis method of MacDonald and Pitcher (1979). Modes are interpreted as representing 1 (mode I), 2 (mode II), 3 (mode III), or 4 or more (mode IV) accumulated ejaculates. The mg-equivalent mean value of each mode appears below the roman numerals.



Table 4

Proportion of primiparous female snow crabs (in boldface) attributed to each of 4 modes identified in frequency distributions of spermathecal loads (see Fig. 4) and mean spermathecal load for each mode (mg, in parentheses) in replicated sex ratio treatments of the 1997 and 1998 mating experiments

Modes	Treatments					
	1997			1998		
	2♂:30♀	2♂:20♀	2♂:10♀	2♂:20♀	4♂:20♀	6♂:20♀
I	<b>0.13</b> (17.1)	<b>0.12</b> (20.4)	<b>0.00</b> (-)	<b>0.11</b> (11.5)	<b>0.19</b> (13.9)	<b>0.00</b> (-)
II	<b>0.72</b> (42.3)	<b>0.74</b> (52.5)	<b>0.28</b> (47.8)	<b>0.76</b> (26.5)	<b>0.41</b> (25.8)	<b>0.71</b> (31.2)
III	<b>0.15</b> (98.2)	<b>0.14</b> (115.4)	<b>0.55</b> (80.2)	<b>0.12</b> (66.6)	<b>0.35</b> (56.3)	<b>0.11</b> (49.9)
IV	<b>0.00</b> (-)	<b>0.00</b> (-)	<b>0.17</b> (255.5)	<b>0.02</b> (182.4)	<b>0.05</b> (116.1)	<b>0.18</b> (86.4)
$\chi^2$	7.10	4.20	2.24	17.52	8.61	12.83
<i>P</i>	0.989	0.980	0.945	0.734	0.929	0.884
<i>n</i>	85	56	31	59	42	39

Goodness of fit ( $\chi^2$  value and probability, *P*) is provided for the multiple-mode model that was adjusted by mixture analysis to spermathecal load frequency distributions for each treatment, following methods of MacDonald and Pitcher (1979). The proportion of total females (*n*) attributed to each mode was not independent of treatment (*G*-test of independence on each year, *P* < 0.05).

well-fertilized clutch) or a large proportion to none of the eggs were fertilized (= poorly fertilized clutch). Failure to fertilize most or all eggs apparently resulted from sperm limitation. Indeed, females with poorly fertilized or well-fertilized clutches had median SL values of 4.4 mg (range: 3.0–107.0 mg, *n* = 9) or 49.6 mg (4.4–438.3 mg, *n* = 145) respectively in 1997 (one-tailed Mann-Whitney test, *U* = 137, *P* < 0.001) and of 3.0 mg (0.0–49.8 mg, *n* = 11) or

30.7 mg (10.2–115.1 mg, *n* = 126) respectively in 1998 (*U* = 174, *P* < 0.001).

No clear relationship between fertilization success and sex ratio was found. The proportion of females with poorly fertilized clutches was slightly greater in 1998 (7.8%) than in 1997 (5.8%) and tended to decline with decreasing sex ratio in each year, but differences between years or among treatments within a year were not significant (*G*-test of independence, *P* > 0.05 for all analyses). However, the proportion of females carrying a poorly fertilized clutch may be underestimated due to the criterion of a postmolt age of 20 d for examination of eggs, which excludes females that molted latest and were more likely to have received small amounts of sperm. This may be especially true of the 2♂:30♀ treatment in 1997 due to the negative correlation between SL and molt date, and of the 1998 experiment overall due to a tardy molting schedule.

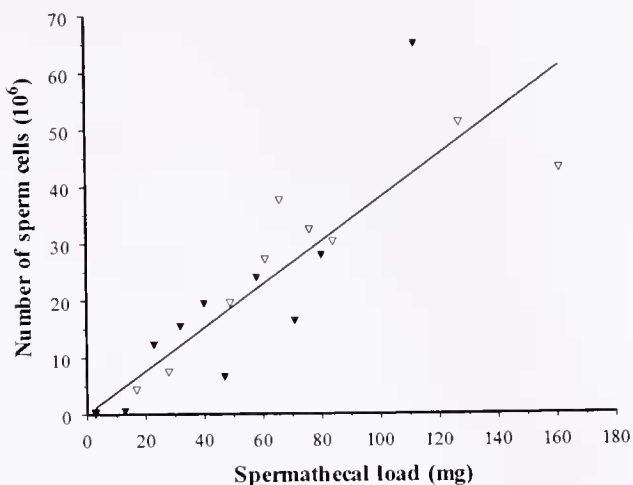


Figure 5. Number of sperm cells (*N*) in relation to the right spermatheca load (SL) of selected primiparous female snow crabs from the 2♂:20♀ (▼) and 10♂:20♀ (▽) treatments of the 1998 mating experiment. A common regression was fitted to the data ( $N = 3.788 \cdot 10^5$  SL, *n* = 20,  $r^2 = 0.91$ , *P* < 0.001).

## Discussion

Molting asynchrony combined with brief periods of peak sexual attractiveness for female snow crabs inflated the operational sex ratio (OSR) in our experiments and led to a context of male competition even when females far outnumbered males (Table 1). As expected in such mating systems, males exhibited flexibility in their allocation of time and sperm to females. Below, we discuss how mate-guarding time, sperm depletion, and sperm expenditure varied in relation to sex ratio and male dominance. We close the

discussion by considering the impact of variable patterns of male mating on female mating success.

#### *Duration of mate guarding*

Male snow crabs reacted to an increasing sex ratio in the 1998 experiment by guarding females longer, in accordance with theory (Grafen and Ridley, 1983; Yamamura and Jormalainen, 1996) and experimental demonstrations in other brachyuran and anomuran crabs (Wilber, 1989; Jivoff, 1997a; Jivoff and Hines, 1998; Wada *et al.*, 1999). Such a behavior had been inferred for snow crab from *in situ* observations that the proportion of premolt females to postmolt females in mating pairs is greater in years of higher than of lower sex ratio (Sainte-Marie *et al.*, 1999).

Due to different assumptions about female choice and male mate-guarding costs and capability to defend or take over mates, models of mate guarding in the Crustacea have predicted that larger males should associate with females for shorter (*e.g.*, Grafen and Ridley, 1983) or longer (*e.g.*, Elwood and Dick, 1990) periods of time than smaller males. In our study, male snow crabs that accumulated the greatest number of guard days were larger, reflecting in part a size advantage for the defense of females and the ability to displace smaller males (Sainte-Marie *et al.*, 1997). The nonproportional decline in the contribution of the dominant male to total guarding days with increasing number of males (Table 3) suggests that slight male advantages may become increasingly important as the intensity of competition escalates. Accordingly, the range of sizes and conditions of males represented in wild mating pairs was narrower when females were relatively scarce than when they were more abundant (Sainte-Marie *et al.*, 1999).

#### *Sperm depletion*

There was clear evidence in our experiments that the sperm reserves of some males were depleted in relation to the number of mating opportunities, as evidenced at the replicate level by progressively smaller mean vasa deferentia weight (VDW) with declining sex ratio (Fig. 1). Moreover, dominant males were significantly more sperm-depleted than subordinate males (Fig. 2), suggesting that the former mated more frequently. This occurred even though the dominant larger males probably had bigger vasa deferentia than the subordinate smaller males at the onset of the experiment, which can be inferred because VDW usually scales positively to male CW (see Sainte-Marie *et al.*, 1995). Part of the difference in VDW with male hierarchy could be due to dominant males charging their vasa deferentia more slowly than subordinate males (see Warner *et al.*, 1995), since energy expenditure and food deprivation may increase with guarding time (Robinson and Doyle, 1985; Sparkes *et al.*, 1996). Furthermore, we posit that the 35% VDW difference in favor of 1997 over 1998 males reflected

sperm depletion through successive breeding periods. Indeed, these males were sampled respectively in the autumn of 1996 and of 1997, 2 and 3 years into a period of intense recruitment of adult females and of declining abundance of large adult males which lasted from 1995 to 1998 (DFO, 2000).

#### *Sperm allocation*

Three findings converge to indicate that male snow crabs allocate sperm parsimoniously and partition it among successive matings, a behavior termed sperm economy (Pitnick and Markow, 1994; Shapiro *et al.*, 1994). First, all primiparous females were submaximally inseminated, as evident from the finding that the largest median SL value of 80 mg in our experiments (Fig. 3) was far less than the record mean value of 256 mg determined for wild primiparous females in a year of intense male competition (Sainte-Marie, 1993). Second, in the 1998 experiment even the largest ejaculates passed to females (72 mg, difference between mean SL for modes IV and III in Fig. 4) represented just 2.3% of VDW of control males, and no male fully exhausted his sperm. Third, spermathecal load (SL) was independent of female molt date in all but the most female-biased treatment (2♂:30♀), indicating that sperm depletion was not the usual cause of reduced female sperm reserves at lower sex ratios.

Sperm economy is predicted by sperm competition theory when females can be polyandrous, mechanisms of last-male sperm precedence can be effective, and the probability that one male fertilizes a female's lifetime production of eggs is small (*e.g.*, Pitnick and Markow, 1994; Parker *et al.*, 1997), all of which are attributes of snow crab. The relatively small size of snow crab ejaculates explains why males can equally inseminate several females in rapid succession (Sainte-Marie and Lovrich, 1994). By contrast, the ejaculates of blue crab (*Callinectes sapidus*) represent on average 47% of male gonad volume (Jivoff, 1997b), indicating a sperm-maximizing strategy that correlates with the typically monandrous behavior of females, or otherwise ineffective sperm-precedence mechanisms, and with the generally high probability that one male fertilizes a female's lifetime production of eggs (see Jivoff, 1997a, b). In blue crab, a severe depletion of sperm reserves occurs after just one mating, and males cannot equally inseminate even two females in rapid succession (Jivoff, 1997b; Kendall and Wolcott, 1999).

In snow crab, the coherent pattern of smaller VDW and SL in the 1998 experiment compared to the 1997 experiment for a given effective sex ratio (ESR) (Figs. 1 and 3) indicates that males with relatively smaller gonads pass less ejaculate than males with relatively larger gonads, and this is further evidence of sperm economy. Furthermore, since mean SLs for corresponding modes were distinctly smaller in 1998 compared to 1997 (Fig. 4), but proportions of

females with 1, 2, 3, or 4 or more ejaculates were nearly identical in the common 2♂:20♀ treatment (Table 4), we conclude that the 55% difference in SL between the two years was due mainly to variation in the size of individual ejaculates.

Superimposed on the pattern of SL set by relative vasa deferentia size, in each year SL increased with increasing sex ratio (Fig. 3). This trend resulted from females accumulating more ejaculates of a progressively larger size with increasing rank of insertion into the spermatheca (Fig. 3, Table 4), and it occurred whether sex ratio was controlled by varying the number of females or males. The greater number of ejaculates reflects some combination of more frequent repeated matings (this is certain in 1997, because only two males were used across all treatments) and multiple matings with growing intensity of male competition. The importance of repeated mating relative to polyandry in providing females with larger sperm stores, especially in the 1998 experiment where the number of males was varied across the treatments, will be resolved by genetic analyses using hypervariable microsatellite DNA. Furthermore, the possibility remains that some measure of the variation of SL in relation to sex ratio was due to males adjusting the size of individual ejaculates with changing intensity of competition.

The greater sperm expenditure at higher sex ratios observed in snow crab represents a widespread response of males to the risk of sperm competition (Gage, 1991; Gage and Barnard, 1996; Jivoff, 1997b; Wedell and Cook, 1999). Moreover, the fact that ejaculate size increased with rank of insertion into the spermatheca (Fig. 4) is consistent with predictions and observations for other species that males expend more sperm with previously inseminated females than with virgin females (Cook and Gage, 1995; Jivoff, 1997a; Parker *et al.*, 1997). Increasing the number or size of ejaculates may represent a swamping strategy in species where sperm mixing occurs and all sperm may potentially access eggs (Pitnick and Markow, 1994). However, sperm stratification occurs within the spermathecae of snow crab, and the advantage of introducing a larger ejaculate may be that it will more effectively displace and isolate any previously deposited sperm away from the ovary efferent duct (Sainte-Marie *et al.*, 2000).

The fact that both guarding time and SL were usually independent of female molt date strongly suggests that from the onset of the experiment male snow crabs adopted complementary mate-guarding and sperm-allocation strategies that remained fixed in time. As proposed by Wada *et al.* (1999) for the hermit crab *Pagurus middendorffii*, the rate at which a male encounters females and other males may provide information on the sociosexual context—that is, the potential number of matings to be realized and the degree of male rivalry—that determines in part the male's mating strategy. Similarly, Vepsäläinen and Savolainen (1995)

demonstrated that past OSR experience could condition future male mating behavior in the water strider *Gerris lacustris*. A mate-guarding and sperm-allocation strategy that was established early in the breeding season in reflection of a male's sperm reserves and dominance rank, and of sociosexual context, would allow the male to maximize the number of females that he inseminated. Such a strategy may be maintained even at the expense of reduced fertilization rate per mating (= sperm limitation) "because it is less costly to the male than becoming sperm-depleted before mating opportunities have ceased" (Warner *et al.*, 1995).

#### *Female mating success and sperm limitation*

Increasing male sexual competition had both positive and negative effects on female mating success. On one hand, SL increased with increasing sex ratio (Fig. 3); this implies that females had progressively more sperm in storage, given the positive relationship between sperm counts and SL in this study (Fig. 5) as in Sainte-Marie and Lovrich (1994). On the other hand, increasing male competition had adverse effects on the post-mating condition and survival of females. The number of missing pereopods per primiparous female (Table 2) and the percent mortality of the fragile nulliparous and primiparous females increased as the number of males and the sex ratio increased. In the present study, the frequency of injury and mortality may to some extent have been amplified by confinement in the tanks. However, there is field evidence that the number of missing pereopods for primiparous females may vary in relation to the intensity of male competition (Sainte-Marie *et al.*, 1999) and that females are killed by fighting males (Sainte-Marie and Hazel, 1992). Moreover, a negative relationship between female fecundity and sex ratio was seen in the 1997 experiment ( $P < 0.05$ ) and also in the 1998 experiment, although the trend was not significant in the latter (Rondeau, 2000). This decline in fecundity is attributed to the loss of recently extruded, weakly attached eggs during interactions between post-oviposition females and males, which may occur more frequently as male bias in sex ratio increases. These negative effects of male mating activities on female fitness constitute another example of intersexual conflict (Rowe *et al.*, 1994; Vepsäläinen and Savolainen, 1995; Jormalainen, 1998).

Snow crab females incubating a poorly fertilized clutch were apparently sperm-limited, since they had SLs one order of magnitude smaller than those of females incubating a well-fertilized clutch. Similarly, using a subjective index of SL (none, small, or large) on wild female snow crabs, Carrière (1995) found that the proportion of females with well-fertilized clutches increased significantly with extent of spermatheca fullness. In our study, however, there were a few cases where females had a relatively large SL yet a small or null proportion of fertilized eggs. This apparent



inconsistency could arise if a female was mated by another male, after initially mating and laying eggs with a dominant male that was particularly sparing of his sperm.

Sperm limitation occurs naturally when males (i) are too few to inseminate all receptive females, (ii) allocate their sperm too parsimoniously among females, or (iii) do not have time to recharge between matings (Pitnick, 1993; Pitnick and Markow, 1994; Warner *et al.*, 1995; Jivoff, 1997b; MacDiarmid and Butler, 1999). For snow crab, the general cause of sperm limitation was probably sperm economy and in the case of the 30♀:2♂ treatment, additionally, perhaps sperm depletion. Small SLs resulting from unfavorable mating conditions during the female's first breeding season may have negative impacts on her subsequent reproductive activities. Size-fecundity relationships for multiparous (= repeat spawners) females (Sainte-Marie, 1993) and the equation relating sperm counts to SL (Fig. 5) allow estimation of the minimum doubled SL value required for fertilization of a second egg clutch using sperm stored over from a previous breeding period. This value is determined considering that an average of 70 sperm cells are expended to fertilize each oocyte (Sainte-Marie and Lovrich, 1994; Yamasaki *et al.*, 1994). On this basis, 5.2% (1997) and 9.2% (1998) of females did not have enough stored sperm to produce (without re-mating) a second clutch with all eggs fertilized. These are necessarily conservative estimates because mortality of stored sperm may occur between ovipositions (Paul, 1984; Sainte-Marie and Sainte-Marie, 1999). Females with insufficient sperm stores will produce fewer or no fertilized eggs, or they will re-mate at the risk of injury or death (Elnor and Beninger, 1995).

In closing, our study has shown that the mating strategies of male snow crabs are quite flexible, which is adaptive to the widely varying levels of competition intensity and female availability that characterize this species. Our study also points to the potential for sperm limitation to occur in exploited snow crab populations if the removal rates of large males are too high. Indeed, fishing will depress the sex ratio and deplete the most competitive component of the male population. As a result of reduced sexual competition the surviving large males may be subject to sperm depletion through extensive mating, which will exacerbate their sperm-economy behavior. Thus, by contrast to the predominant view in crab fisheries literature that sperm limitation could arise from the number of males becoming insufficient to service all females (see Kruse, 1993; Elnor and Beninger, 1995), the present study revealing the sperm-economy behavior of male snow crabs suggests an insidious process of suboptimal insemination. Further research will consider the implications of sperm economy for the conservation and management of snow crab.

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## Literature Cited

- Adams, A. E., and A. J. Paul. 1983. Male parent size, sperm storage and egg production in the crab *Chionoecetes bairdi* (Decapoda, Majidae). *Int. J. Invertebr. Reprod.* 6: 181–187.
- Alunno-Bruscia, M., and B. Sainte-Marie. 1998. Abdomen allometry, ovary development, and growth of female snow crab *Chionoecetes opilio* in the Gulf of Saint Lawrence (Brachyura, Majidae). *Can. J. Fish. Aquat. Sci.* 55: 459–477.
- Bouchard, S., B. Sainte-Marie, and J. N. McNeil. 1996. Indirect evidence indicates female semiochemicals release male precopulatory behaviour in the snow crab, *Chionoecetes opilio* (Brachyura: Majidae). *Chemoecology* 7: 39–44.
- Carrière, C. 1995. Insémination et fécondité chez la femelle du crabe des neiges *Chionoecetes opilio* de l'estuaire maritime du Saint-Laurent. Master's thesis, Université du Québec à Rimouski, Rimouski, Canada. 88 pp.
- Christy, J. H. 1987. Competitive mating, mate choice and mating associations of brachyuran crabs. *Bull. Mar. Sci.* 41: 177–191.
- Clutton-Brock, T. H., and G. A. Parker. 1992. Potential reproductive rates and the operation of sexual selection. *Q. Rev. Biol.* 4: 437–456.
- Cook, P. A., and M. J. G. Gage. 1995. Effects of risk of sperm competition on the numbers of eupyrene and apyrene sperm ejaculated by the moth *Plodia interpunctella* (Lepidoptera: Pyralidae). *Behav. Ecol. Sociobiol.* 36: 261–268.
- DeGoursey, R. E., and P. J. Auster. 1992. A mating aggregation of the spider crab (*Libinia emarginata*). *J. Northwest Atl. Fish. Sci.* 13: 77–82.
- Dewsbury, D. A. 1982. Ejaculate cost and male choice. *Am. Nat.* 119: 601–610.
- DFO (Department of Fisheries and Oceans, Canada). 2000. Snow crab of the Estuary and Northern Gulf of St. Lawrence (areas 13 to 17). *DFO Science Stock Status Rep.* C4-01 (2000). 13 pp.
- Donaldson, W. E., and A. E. Adams. 1989. Ethogram of behavior with emphasis on mating for the Tanner crab *Chionoecetes bairdi* Rathbun. *J. Crustac. Biol.* 9: 37–53.
- Elnor, R. W., and P. G. Beninger. 1995. Multiple reproductive strategies in snow crab, *Chionoecetes opilio*: physiological pathways and behavioral plasticity. *J. Exp. Mar. Biol. Ecol.* 193: 93–112.
- Elwood, R. W., and J. T. A. Dick. 1990. The amorous *Gammarus*: the relationship between precopula duration and size-assortative mating in *G. pulex*. *Anim. Behav.* 39: 828–833.
- Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* 197: 215–223.
- Gage, A. R., and C. J. Barnard. 1996. Male crickets increase sperm number in relation to competition and female size. *Behav. Ecol. Sociobiol.* 38: 349–353.
- Gage, M. J. G. 1991. Risk of sperm competition directly affects ejaculate size in the Mediterranean fruit fly. *Anim. Behav.* 42: 1036–1037.
- Grafen, A., and M. Ridley. 1983. A model of mate guarding. *J. Theor. Biol.* 102: 549–567.

- Hartnoll, R. G. 1969. Mating in the Brachyura. *Crustaceana* **16**: 161–181.
- Jivoff, P. 1997a. The relative roles of predation and sperm competition on the duration of the post-copulatory association between the sexes in the blue crab, *Callinectes sapidus*. *Behav. Ecol. Sociobiol.* **40**: 175–186.
- Jivoff, P. 1997b. Sexual competition among male blue crab, *Callinectes sapidus*. *Biol. Bull.* **193**: 368–380.
- Jivoff, P., and A. H. Hines. 1998. Effect of female molt stage and sex ratio on courtship behavior of the blue crab *Callinectes sapidus*. *Mar. Biol.* **131**: 533–542.
- Jormalainen, V. 1998. Precopulatory mate guarding in crustaceans: male competitive strategy and intersexual conflict. *Q. Rev. Biol.* **73**: 275–304.
- Kendall, M. S., and T. G. Wolcott. 1999. The influence of male mating history on male-male competition and female choice in mating associations in the blue crab, *Callinectes sapidus* (Rathbun). *J. Exp. Mar. Biol. Ecol.* **239**: 23–32.
- Kruse, G. H. 1993. Biological perspectives on crab management in Alaska. Pp. 355–384 in *International Symposium on Management Strategies for Exploited Fish Populations*, B. Baxter, ed. Lowell Wakefield Fisheries Symposium Series, University of Alaska Fairbanks, Alaska Sea Grant College Program Report 93-02.
- MacDiarmid, A. B., and M. J. Butler. 1999. Sperm economy and limitation in spiny lobsters. *Behav. Ecol. Sociobiol.* **46**: 14–24.
- MacDonald, P. D. M., and T. J. Pitcher. 1979. Age-groups from size-frequency data: a versatile and efficient method of analysing distribution mixtures. *J. Fish. Res. Board Can.* **36**: 987–1001.
- Moriyasu, M., and C. Lanteigne. 1998. Embryo development and reproductive cycle in the snow crab, *Chionoecetes opilio* (Crustacea: Majidae), in the southern Gulf of St. Lawrence, Canada. *Can. J. Zool.* **76**: 2040–2048.
- Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**: 525–567.
- Parker, G. A. 1974. Courtship persistence and female-guarding as male time investment strategies. *Behaviour* **48**: 157–184.
- Parker, G. A., M. A. Ball, P. Stockley, and M. J. G. Gage. 1997. Sperm competition games: a prospective analysis of risk assessment. *Proc. R. Soc. Lond. B* **264**: 1793–1802.
- Paul, A. J. 1984. Mating frequency and viability of stored sperm in the Tanner crab *Chionoecetes bairdi* (Decapoda, Majidae). *J. Crustac. Biol.* **4**: 375–381.
- Paul, A. J., and A. E. Adams. 1984. Breeding and fertile period for female *Chionoecetes bairdi* (Decapoda, Majidae). *J. Crustac. Biol.* **4**: 589–594.
- Pelletier, N., A. Fraser, D. Gauthier, M. Lavolette, and M. Moriyasu. 1998. Mise en œuvre d'une méthode pour l'analyse biochimique du mécanisme d'accouplement chez le crabe des neiges (*Chionoecetes opilio*). *Rapp. Tech. Can. Sci. Halieut. Aquat.* **2200**. 25 pp.
- Pitnick, S. 1993. Operational sex ratios and sperm limitation in populations of *Drosophila pachea*. *Behav. Ecol. Sociobiol.* **33**: 383–391.
- Pitnick, S., and T. A. Markow. 1994. Male gametic strategies: sperm size, testes size, and the allocation of ejaculate among successive mates by the sperm-limited fly *Drosophila pachea* and its relatives. *Am. Nat.* **143**: 785–819.
- Ridley, M. 1983. *The Explanation of Organic Diversity. The Comparative Method and Adaptations for Mating*. Clarendon Press, Oxford. 272 pp.
- Robinson, B. W., and R. W. Doyle. 1985. Trade-off between male reproduction (amplexus) and growth in the amphipod *Gammarus lawrencianus*. *Biol. Bull.* **168**: 482–488.
- Rondeau, A. 2000. Économie de sperme par les mâles et succès reproducteur des femelles primipares chez le crabe des neiges, *Chionoecetes opilio*, en fonction du contexte sexuel. Master's thesis, Université du Québec à Rimouski, Rimouski, Canada. 101 pp.
- Rowe, L., G. Arnqvist, A. Sih, and J. J. Krupa. 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends Ecol. Evol.* **9**: 289–293.
- Sainte-Marie, B. 1993. Reproductive cycle and fecundity of primiparous and multiparous female snow crab, *Chionoecetes opilio*, in the north-west Gulf of Saint Lawrence. *Can. J. Fish. Aquat. Sci.* **50**: 2147–2156.
- Sainte-Marie, B., and C. Carrière. 1995. Fertilization of the second clutch of eggs of snow crab, *Chionoecetes opilio*, from females mated once or twice after their molt to maturity. *Fish. Bull. (Seattle)* **93**: 759–764.
- Sainte-Marie, B., and F. Hazel. 1992. Moulting and mating of snow crabs, *Chionoecetes opilio* (O. Fabricius), in shallow waters of the northwestern Gulf of Saint Lawrence. *Can. J. Fish. Aquat. Sci.* **49**: 1282–1293.
- Sainte-Marie, B., and G. A. Lovrich. 1994. Delivery and storage of sperm at first mating of female *Chionoecetes opilio* (Brachyura: Majidae) in relation to size and morphometric maturity of male parent. *J. Crustac. Biol.* **14**: 508–521.
- Sainte-Marie, G., and B. Sainte-Marie. 1999. Reproductive products in the adult snow crab (*Chionoecetes opilio*). II. Multiple types of sperm cells and of spermatophores in the spermathecae of mated females. *Can. J. Zool.* **77**: 451–462.
- Sainte-Marie, B., S. Raymond, and J.-C. Brêthes. 1995. Growth and maturation of the benthic stages of male snow crab, *Chionoecetes opilio* (Brachyura: Majidae). *Can. J. Fish. Aquat. Sci.* **52**: 903–924.
- Sainte-Marie, B., J.-M. Sévigny, B. D. Smith, and G. A. Lovrich. 1996. Recruitment variability in snow crab (*Chionoecetes opilio*): pattern, possible causes, and implications for fishery management. Pp. 451–478 in *Proceedings of the International Symposium on Biology, Management, and Economics of Crabs from High Latitude Habitats*, B. Baxter, ed. Lowell Wakefield Fisheries Symposium Series, University of Alaska Fairbanks, Alaska Sea Grant College Program Report 96-02.
- Sainte-Marie, B., J.-M. Sévigny, and Y. Gauthier. 1997. Laboratory behavior of adolescent and adult males of the snow crab (*Chionoecetes opilio*) (Brachyura: Majidae) mated noncompetitively and competitively with primiparous females. *Can. J. Fish. Aquat. Sci.* **54**: 239–248.
- Sainte-Marie, B., N. Urbani, J.-M. Sévigny, F. Hazel, and U. Kuhnlein. 1999. Multiple choice criteria and the dynamics of assortative mating during the first breeding season of female snow crab *Chionoecetes opilio* (Brachyura, Majidae). *Mar. Ecol. Prog. Ser.* **181**: 141–153.
- Sainte-Marie, G., B. Sainte-Marie, and J.-M. Sévigny. 2000. Ejaculate-storage patterns and the site of fertilization in female snow crabs (*Chionoecetes opilio*: Brachyura, Majidae). *Can. J. Zool.* **78**: 1902–1917.
- Shapiro, D. Y., A. Marconato, and T. Yoshikawa. 1994. Sperm economy in a coral reef fish. *Thalassoma bifasciatum*. *Ecology* **75**: 1334–1344.
- Smith, R. L., ed. 1984. *Sperm Competition and the Evolution of Animal Mating Systems*. Academic Press, New York. 661 pp.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry*, 3rd ed. W. H. Freeman, New York. 887 pp.
- Sparkes, T. C., D. P. Keogh, and R. A. Pary. 1996. Energetic costs of mate guarding behavior in male stream dwelling isopods. *Cecologia* **106**: 166–171.
- Stevens, B. G., W. E. Donaldson, J. A. Haaga, and J. E. Munk. 1993. Morphometry and maturity of paired Tanner crabs, *Chionoecetes bairdi*, from shallow- and deepwater environments. *Can. J. Fish. Aquat. Sci.* **50**: 1504–1516.
- Stevens, B. G., J. A. Haaga, and W. E. Donaldson. 1994. Aggregative mating of Tanner crabs, *Chionoecetes bairdi*. *Can. J. Fish. Aquat. Sci.* **51**: 1273–1280.
- Trivers, R. L. 1972. Parental investment and sexual selection. Pp. 136–

- 179 in *Sexual Selection and the Descent of Man, 1871–1971*. B. Campbell, ed., Aldine Press, Chicago.
- Urbani, N., B. Sainte-Marie, J.-M. Sévigny, D. Zadworny, and U. Kuhnlein. 1998.** Sperm competition and paternity assurance during the first breeding period of female snow crab *Chionoecetes opilio* (Brachyura: Majidae). *Can. J. Fish. Aquat. Sci.* **55**: 1104–1113.
- Vepsäläinen, K., and R. Savolainen. 1995.** Operational sex ratios and mating conflicts between the sexes in the water strider *Gerris lacustris*. *Am. Nat.* **146**: 869–880.
- Wada, S., K. Tanaka, and S. Goshima. 1999.** Precopulatory mate guarding in the hermit crab *Pagurus middendorffii* (Brandt) (Decapoda: Paguridae): effects of population parameters on male guarding duration. *J. Exp. Mar. Biol. Ecol.* **239**: 289–298.
- Warner, R. R., D. Y. Shapiro, A. Marcanato, and C. W. Petersen. 1995.** Sexual conflict: males with the highest mating success convey the lowest fertilization benefits to females. *Proc. R. Soc. Lond. B* **262**: 135–139.
- Watson, J. 1972.** Mating behavior in the spider crab, *Chionoecetes opilio*. *J. Fish. Res. Board Can.* **29**: 447–449.
- Wedell, N., and P. A. Cook. 1999.** Butterflies tailor their ejaculate in response to sperm competition risk and intensity. *Proc. R. Soc. Lond. B* **266**: 1033–1039.
- Wilber, D. II. 1989.** The influence of sexual selection and predation on the mating and postcopulatory guarding behavior of stone crabs (Xanthidae, *Menippe*). *Behav. Ecol. Sociobiol.* **24**: 445–451.
- Yamamura, N., and V. Jormalainen. 1996.** Compromised strategy resolves intersexual conflict over precopulatory guarding duration. *Evol. Ecol.* **10**: 661–680.
- Yamasaki, A., S. Fujita, K. Uchino, and T. Toshima. 1994.** Research into the ecology of snow crabs in the waters off Kyoto Prefecture—IX. Numbers of sperm in the spermathecas of female snow crabs. *Res. Rep. Kyoto Inst. Ocean Fish. Sci.* **17**: 19–24 (*Can. Transl. Fish. Aquat. Sci.* 5663).